

## MINI REVIEW

## Taxonomy of the Truffles

Samad Jamali

## Abstract

**Truffles are the hypogeous fruiting bodies of Ascomycete fungi that live in symbiosis with roots of trees such as oaks, hazels, poplar, etc. Due to limited morphological characters, these fungi are difficult to identify at species level. Molecular phylogenetic studies have recently demonstrated that morphological characters of hypogeous Ascomycetes can be unreliable.**

**Keywords:** truffles; ascomycetes; fungi

## Introduction

Truffles can be divided into two types: tuber and desert truffles. Truffles are the hypogeous fruiting bodies of Ascomycete fungi that live in symbiosis with the roots of trees such as oaks, hazels, poplar (Harley & Smith, 1983), some shrubs as *Cistus*, members of *Cistaceae*, mainly with *Helianthemum* species, both annual and perennial (Awameh, Alsheikh, & Al-Ghawwas, 1979; Honrubia, Cano, & Molina-Ninriola, 1992; Morte, Lovisolo, & Schubert, 2000; Kovacs, Bagi, Vagvolgy, Kottke, & Oberwinkler, 2003), and members of *Cypraceae* (Ammarellou, Saremi, & Gucin, 2007; Jamali & Banihashemi, 2012, 2013). Nowadays the taxonomical criteria to identify truffles are size and shape of spores and asci, spore wall ornamentation and structure of the peridium and gleba (Mello, Murat, & Bonfante, 2006). Due to their limited morphological characteristics,

these fungi are difficult to identify at species level. Molecular phylogenetic studies have recently demonstrated that morphological characteristics of hypogeous Ascomycetes can be unreliable (Ferdman, Aviram, Roth-Bejerano, Trappe, & Kagan-Zur, 2005). Other molecular studies of truffles have led to the reclassification of some genera or species (O'Donnell, Cigelnik, Weber, & Trappe, 1997; Hansen, Lassoë, & Pfister, 2001).

## Traditional taxonomy

Traditional taxonomy is based on external or internal morphological criteria. Vittadini (1831) was the first to introduce such a classification of truffles and distinguished two groups based on their texture and organoleptic properties. In 1862 the Tulasne brothers published a treatise on truffles classifying them based on microscopic criteria. From the 18<sup>th</sup> to the 20<sup>th</sup> century, about 200 species, varieties and forms of truffles were described by mycologists of various countries (Mello *et al.*, 2006). Limited communication means during that time introduced many redundancies so that this number was recently reduced by Ceruti, Fontana, & Nosenzo, (2003) who considered only 28 species to be valid for Europe. Worldwide the number of truffle species is estimated to be around 60 (Trappe, 1979). Morphological characteristics have been used to describe different species of truffles including size and shape of spores and asci, spore wall ornamentation, structure of the peridium and gleba and organoleptic characteristics (Trappe, 1979; Mello *et al.*, 2006). In 1972, a synoptic key for the *Pezizales* provided the means to discern the relationship between genera of *Tuberales* and genera of *Pezizales* (Korf, 1972). The key contains six major categories of character: ascocarp morphology, peridium, asci, paraphyses, spores and habitat. These criteria are however not sufficient for species with very similar morphological features and that are difficult to identify at the species level (Diez, Manjon, & Martin, 2002). Biophysical (Papa, Balbi, & Ausidio, 1987), biochemical (Mouches, Duthil, Poitou, Delmas, & Bove, 1981; Dupre & Chevalier, 1991; Palenzona *et al.*, 1990; Cameleyre & Olivier, 1993; Gandeboeuf, Dupre, & Chevalier, 1994), olfactory (Pacioni & Pomponi, 1989), and immunological (Corocher, Polimeni, Giraudi, & Papa, 1992;

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Neuner-Plattner *et al.*, 1999) tools have been developed in the past to identify and characterize truffle species, but have since been superseded by faster and easier molecular techniques. Also, their use to specifically identify mycorrhiza is limited by the amount of biological material required. In contrast, PCR-based DNA detection can be performed on minute amounts of tissue.

### Molecular Taxonomy

Desert truffles were traditionally grouped into the *Terfeziaceae* family of fungi, within the order *Tuberales*. In recent years, molecular phylogenetic studies demonstrated that the morphological characteristics of hypogeous Ascomycetes can be misleading (Ferdman *et al.*, 2005) due to a reduction in the macromorphological characteristics needed for distinguishing their related epigeous taxa. Thus O'Donnell *et al.*, (1997) and Hansen *et al.*, (2001) demonstrated that certain hypogeous fungi show greater affinity to epigeous members of the *Pezizales* than to other hypogeous species. It seems that on at least 15 independent occasions epigeous fungi within the *Pezizales* have evolved the below-ground fruiting mechanisms required to become truffles (Hansen, 2001). Other molecular studies on truffles have led to the reclassification of some genera or species (O'Donnell *et al.*, 1997; Norman & Egger, 1999; Percudani *et al.*, 1999; Roux *et al.*, 1999; Hansen *et al.*, 2001 and Diez *et al.*, 2002). Some of these investigations confirm earlier morphological findings. Of 31 genera of hypogeous Ascomycotina accepted, one is assigned to the monotypic order *Elaphomycetales* and the rest are placed in the *Pezizales*. The polyphyletic order *Tuberales* is abandoned. Nine families of the *Pezizales* include hypogeous species: *Helvellaceae*, *Pezizaceae*, *Ascobolaceae*, *Pyronemataceae*, *Balsamiaceae*, *Geneaceae*, *Terfeziaceae*, *Tuberaceae* and *Garbomycetaceae* (Trappe, 1979). The hypogeous ascomycete fungi (truffles) are now distributed among six *Pezizalean* families: *Glaziellaceae*, *Discinaceae-Morchellaceae*, *Helvellaceae*, *Tuberaceae*, *Pezizaceae*, and *Pyronemataceae*, comprising 38 genera (Hansen *et al.*, 2001). The family *Tuberaceae*, which includes the most highly prized (and priced) forest truffles is the single family containing only underground species (Hansen *et al.*, 2001). The best known and appreciated genera *Terfezia* and *Tirmania* were shown to belong to the *Pezizaceae* rather than to the distinctly hypogeous *Terfeziaceae* family, which has been abolished (Norman & Egger, 1999; Percudani *et al.*, 1999). Similarly, the monophyletic origin of some members of *Terfezia* and *Tirmania* was confirmed by Diez *et al.*, (2002). The genus *Choiromyces* was transferred from the *Pezizaceae* to the *Tuberaceae* (O'Donnell *et al.* 1997, Percudani *et al.* 1999), although one *Choiromyces* species, *C. echinulatus*, was removed from this genus and restored to the *Pezizaceae* under a new name, *Eremiomyces echinulatus* (Ferdman *et al.*, 2005). Similarly, two species were removed from the

*Terfezia* genus: *Terfezia terfezioides*, reinstated as *Mattirolomyces terfezioides* (Percudani *et al.*, 1999; Diez *et al.*, 2002) and *Terfezia pfeilii*, renamed *Kalaharituber pfeilii* (Ferdman *et al.*, 2005). Such molecular data are not yet available for *Mycoclelandia* (an Australian desert genus with two species), but it was assigned to the *Pezizaceae* because of its strongly amyloid asci (Trappe, 1979; Trappe & Beaton, 1984). Cryptic species (truffles that are morphologically identical but molecularly distinct and distant from each other) are coming to light, including a group of three discovered within the *T. boudieri* desert truffle complex (Ferdman *et al.*, 2005). The *Pezizaceae* includes (among others) the following hypogeous genera: *Eremiomyces*, *Hydnotryopsis*, *Kalaharituber*, *Mattirolomyces*, *Pachyphloeus*, *Peziza*, *Ruhlandiella*, *Stephensia*, *Terfezia*, and *Tirmania* (Laessøe & Hansen, 2007). Of these *Terfezia* is the held in the highest regard for eating; followed by *Kalaharituber*, *Tirmania*, and *Mattirolomyces*.

### Molecular tools for identification of truffles

Much of the molecular research aimed at identifying fungi in general (Gardes, White, Fortin, Bruns, & Taylor, 1991; Henrion *et al.*, 1992; Buscot *et al.*, 1996; Mello *et al.*, 1996; Chillali, Wipf, Guillaumin, Mohammed, & Botton, 1998) and truffles in particular (Henrion *et al.*, 1994; Paolocci, Angelini, Cristofari, Granetti, & Arcioni, 1995; Paolocci, Rubini, Granetti, & Arcioni, 1999) relies on comparison of sequences and RFLP patterns of the ITS region. Several phylogenetic studies of truffles have also used this DNA region (Roux *et al.*, 1999; Diez *et al.*, 2002). Other molecular phylogenetic studies have been based on sequences from the 18S and the 28S (LSU) rRNA genes with or without the ITS region (e.g. O'Donnell *et al.*, 1997; van Tuinen, Jacquot, Zhao, Gollotte, & Gianinazzi-Pearson, 1998; Norman & Egger, 1999; Percudani *et al.*, 1999; Hansen *et al.*, 2001). In recent years, several studies have focused on truffle phylogeny using several loci, and these have allowed the main species to be clearly defined (Halasz *et al.*, 2005; Mello *et al.*, 2005; Paolocci, Rubini, Riccioni, Topini, & Arcioni, 2004; Wang *et al.*, 2006; Weden, Danell, & Tibell, 2005; Zhang, Yang, & Song, 2005). As an example, Wang *et al.* (2006) investigated the European and Asian truffle phylogeny by sequencing several genomic loci. The intraspecific ITS variability in *T. magnatum*, *T. melanosporum*, *T. mesentericum*, *T. aestivum*, and *T. indicum* has been widely investigated (Mello *et al.*, 2005; Murat *et al.*, 2004; Paolocci *et al.*, 2004; Wang *et al.*, 2006; Weden *et al.*, 2005; Zhang *et al.*, 2005), and it has been revealed that the most expensive species (*T. magnatum* and *T. melanosporum*) have a low level of ITS variability. On the other hand, *T. mesentericum*, *T. aestivum*, and *T. indicum* showed a higher diversity level. ITS regions are present in multiple copies and tend to be similar within and variable between fungal species. Species-specific primers have been designed for several

*Tuber* species by analyzing the ITS region (Amicucci, Zambonelli, Giomaro, Potenza, & Stocchi, 1998; Henrion *et al.*, 1994; Paolocci *et al.*, 1995, 1997; Rubini *et al.*, 1998) and protocols for extraction of DNA from fruitbodies have been optimized (Sejalón-Delmas *et al.*, 2000). Other PCR-based methods have also been used: RAPD (Gandeboeuf *et al.*, 1997), microsatellites (Lanfranco, Wyss, Marzachi, & Bonfante, 1993), and the corresponding primers specifically designed. The rRNA genes present different levels of conservation which can be exploited to analyze any desired phylogenetic level (Hillis & Dixon, 1991). Regarding the systematics of desert truffles, the use of morphological features is problematic, because of the reduced set of morphological characteristics and their homoplasy. Ascocarp features are homoplastic as a result of parallel evolution of independent lineages of epigeous/hypogeous fruit bodies during the evolutionary history of the *Pezizales* (Trappe, 1979).

## Conclusion

Morphological characters have been used to describe different species of truffles. Due to limited morphological characters, these fungi are difficult to identify at species level. Molecular phylogenetic studies have recently demonstrated that morphological characters of hypogeous Ascomycetes can be unreliable. rDNA contains a mosaic of highly conserved and variable regions that enable inter- and intra-specific comparisons. These genes occur in multiple arrays, and mutations in their noncoding region occur at a rate that approximates the rate of species emergence. Over time, such mutations become fixed through unequal crossing over and gene conversion, a process that is commonly termed concerted evolution. Within a species ITS tend to be distinct and monomorphic. They, therefore, are suitable for species discrimination across a wide range of organisms including truffles and other fungi.

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